thereof, having similar or better binding affinity as the CDR donor variable region. As described above, the grafting is accomplished by generating a diverse library of CDR grafted variable region fragments and then screening the library for binding activity similar or better than the binding activity of the donor. However, the diverse library is generated by selecting acceptor framework positions that are predicted to affect CDR binding affinity and making a library population containing of all possible amino acid residue changes at each of those positions or subsets of the selected amino acid positions together with all possible amino acid residue changes at each position within the CDRs of the variable region, or subsets of CDR positions. The grafting is accomplished by splicing a population of encoding nucleic acids for the donor CDR containing the selected position changes into a population of encoding nucleic acids for an antibody acceptor variable region framework which contains the selected position changes.

In yet another embodiment, the invention is directed to the optimization of binding affinity of an antibody variable region. The optimization is accomplished by generating a library of variable regions which contain all possible amino acid residue changes at each amino acid position within two or more CDRs. When expressed and screened for binding activity, the variable region, or heavy and light chain heteromeric binding fragments, those species within the population are selected that contain increased or decreased binding activity compared to the parent molecule as optimal binders. Libraries containing subsets, representing less than all amino acid positions within the CDRs, can similarly be generated and screened for selecting optimal

binding variable regions and heteromeric binding fragments thereof.

As used herein, the term "CDR" or "complementarity determining region" is intended to mean the non-contiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. These particular regions have been described by Kabat et al., J. Biol. Chem. 252, 6609-6616 (1977) and Kabat et al., *Sequences of protein of immunological interest.* (1991) , and by Chothia et al., J. Mol. Biol. 196:901-917 (1987) and by MacCallum et al., J. Mol. Biol. 262:732-745 (1996) where the definitions include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or grafted antibodies or variants thereof is intended to be within the scope of the term as defined and used herein. The amino acid residues which encompass the CDRs as defined by each of the above cited references are set forth below in Table 1 as a comparison.

Table 1: CDR Definitions

	Kabat[1]	Chothia[2]	MacCallum[3]
V_H CDR1	31-35	26-32	30-35
V_H CDR2	50-65	53-55	47-58
V_H CDR3	95-102	96-101	93-101
V_L CDR1	24-34	26-32	30-36
V_L CDR2	50-56	50-52	46-55
V_L CDR3	89-97	91-96	89-96

[1] Residue numbering follows the nomenclature of Kabat et al., *supra*

[2] Residue numbering follows the nomenclature of Chothia et al., *supra*

[3] Residue numbering follows the nomenclature of MacCallum et al., *supra*

As used herein, the term "framework" when used in reference to an antibody variable region is entered to mean all amino acid residues outside the CDR regions within the variable region of an antibody. Therefore, a variable region framework is between about 100-120 amino acids in length but is intended to reference only those amino acids outside of the CDRs.

As used herein, the term "framework region" is intended to mean each domain of the framework that is separated by the CDRs. Therefore, for the specific example of a heavy chain variable region and for the CDRs as defined by Kabat et al., framework region 1 corresponds to the domain of the variable region encompassing amino acids 1-30; region 2 corresponds to the domain of the variable region encompassing amino acids 36-49; region 3 corresponds to the domain of the variable region encompassing amino acids 66-94, and